ACTIVITY CONTROL IN NITRIFYING BIOFILMS: APPLICATION OF RESPIROMETRIC TESTS

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INTRODUCTION

Nitrification is the biological oxidation of ammonia to nitrate via nitrite. These reactions are performed by aerobic autotrophic microorganisms, commonly known as nitrifiers. The nitrifiers are generally constituted of ammonia oxidizing bacteria and nitrite oxidizing bacteria. They are very sensitive to environmental factors, namely the pH, which has its optimum value in the range of 7.5 to 8.5 (Sharma end Ahlert, 1977)

A simple method, the respiration rate measurement, has been extensively used in literature to characterize microbial populations in suspended cultures (Kristensen *et al.*, 1992, Nowak and Svardal, 1993) and to monitor the nitrification performance of immobilized nitrifiers (Nakamura *et al.*, 1995) and nitrifying activated sludge processes (Surmacz-Gorska *et al.*, 1996). Respiration refers to reactions that use oxygen as an electron acceptor.

The aim of the present study is to characterize the functional microorganism groups of a nitrifying biofilm using a simple microbial activity measurement, the respiration rate, and to use this information to identify disturbances that have an impact on process performance.

MATERIALS AND METHODS

Biofilm
The biofilm used in the present work was obtained from a laboratory scale circulating bed reactor (CBR) operating in tertiary nitrification. Details on the reactor and the conditions of operation have been described elsewhere (Lazarova *et al.*, 1997). Biofilm was sampled during two different operating conditions of the CBR: pH not controlled (ranging from 6.5 to 7.6) and pH controlled at 7.5.

Measuring equipment
A biological oxygen monitor (BOM), was used to determine the respiration rate of the biofilm in short term experiments. The experiments were performed in a Yellow Springs Instruments BOM (Model 53), schematically represented in Fig.1.

The BOM consists of two measuring chambers (1) with dissolved oxygen (DO) probes (2) and a thermostated jacket that enables it to maintain a constant temperature in the measuring chambers. Mixing was provided by magnetic rods (4).

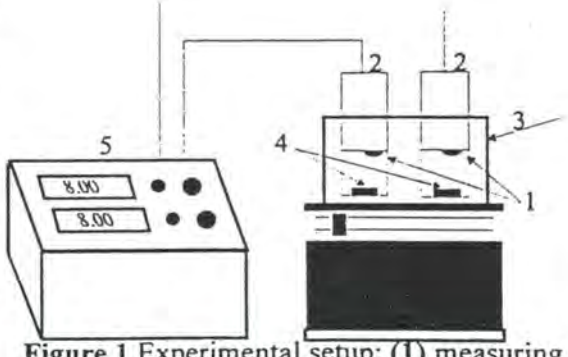

Figure 1 Experimental setup: **(1)** measuring chambers; **(2)** DO probes; **(3)** thermostated jacket; **(4)** magnetic rods; **(5)** oxymeter.

Respiration takes place within the measuring chambers (volume 10 - 12 mL) which are closed free from bubbles by means of tightly fitting stoppers surrounding the DO probes. Each stopper is provided with an injection shaft which allows the pulse addition of chemicals. Oxygen depletion was monitored continuously by an oxymeter (5) and the signal from the probes was measured every second via a LABTECH NOTEBOOK Ltd. (USA) data acquisition card supported with a corresponding software installed in a personal computer.

Experimental procedure
A 1 mL sample of biofilm particles was routinely removed from the CBR reactor to perform the experiments. The latter were carried out at 25 °C with ammonia and nitrite concentrations between 0.5 and 10 mgN.L^{-1}. The biofilm sample was washed with distilled water three times and aerated in a phosphate buffer solution for half an hour to eliminate the residual substrate. Afterwards, the biofilm was placed within the measuring chamber together with 10 mL of phosphate buffer, previously saturated with air. The measuring chamber was closed with the stopper and the decrease of DO concentration as a function of time was monitored. The slope of the linear decrease of DO concentration was assumed to be the endogenous respiration rate of the biofilm. Subsequently, a defined volume of a concentrated substrate solution (ammonium sulphate or potassium nitrite) was injected into the measuring chamber. The slope of the initial linear decrease in the DO concentration after the injection was determined and corresponds to the total respiration rate. The difference between the two respiration rates gives the oxygen consumption rate due to the substrate oxidation. The specific respiration rate was calculated dividing the respiration rate due to substrate oxidation by the biofilm total protein. Each experiment was done in duplicate.

Analytical methods
The biofilm mass was estimated by means of total protein (TP) measured according to the method of Lowry (Lazarova *et al.*, 1994).

RESULTS AND DISCUSSION

The change of the specific respiration rate with ammonia and nitrite bulk concentrations is presented in Fig. 2. The specific respiration rate increased linearly with ammonia concentration up to a value of 2 mgN-NH_4^+.L^{-1}, and then reached maximum constant values of 156 and 432 gO_2.$kgPT^{-1}$.h^{-1} respectively without and with pH control.

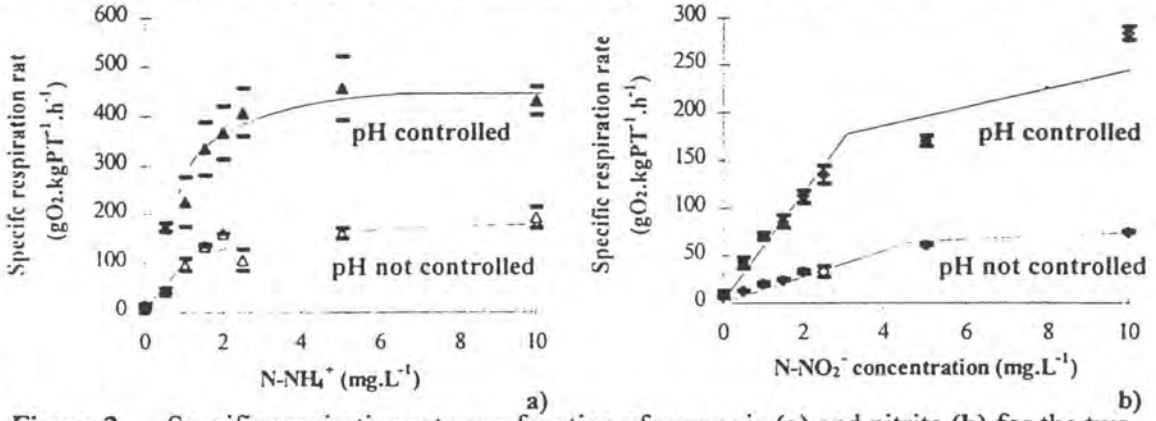

Figure 2. Specific respiration rate as a function of ammonia **(a)** and nitrite **(b)** for the two reactor operating conditions: pH not controlled and pH controlled .

The specific respiration rate due to nitrite oxidation did not reach a maximum constant value for nitrite concentrations in the range of 0.5 to 10 mgN-NO_2^-.L^{-1}. It would be necessary to use higher nitrite concentrations in order to obtain the maximum specific respiration rate.

In Fig. 3 we have the comparison of the maximum specific respiration rate due to ammonia oxidation for the two operating conditions without and with pH control. The pH control implementation results in an increase of the specific respiration rate by a factor of 2.8.

The same figure shows also a comparison of the specific respiration rate for nitrite oxydation for the bulk nitrite concentration of 10 mgN-NO_2^-.L^{-1}. It can be observed an increase on the specific respiration rate by a factor of 3.8 due to pH control.

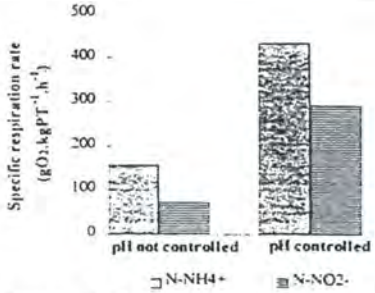

Figure 3. Maximum specific respiration rates for ammonia and nitrite oxidation for the two reactor operating conditions: pH not controlled and pH controlled.

In order to compare the specific ammonia oxidation rate determined in continuous CBR operation with the maximum specific respiration rate obtained in the BOM (Fig. 4), the later was transformed into the specific ammonia oxidation rate with the assumption of total ammonia oxidation (4.57 $gO_2.g^{-1}N\text{-}NH_4^+$). For the case of not controlled pH, the specific ammonia oxidation rate values obtained by both procedures are similar, once the ammonia concentration was not limiting (15 $mgN\text{-}NH_4^+.L^{-1}$ for CBR continuous operation and 10 $mgN\text{-}NH_4^+.L^{-1}$ for the BOM). Due to the pH control implementation, the specific ammonia oxidation rate increased and the ammonia concentration in the CBR became limiting (< 5 $mgN\text{-}NH_4^+.L^{-1}$).

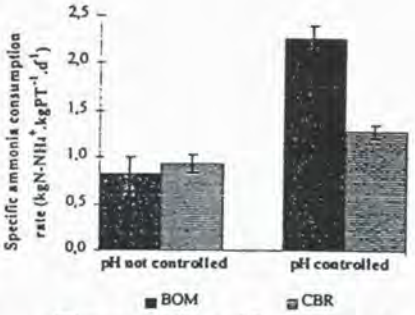

Figure 4. Specific ammonia oxidation rate obtained in CBR continuous operation and determined in the BOM.

This fact explains the large difference between the values obtained in the BOM (where ammonia concentration - 10 $mgN\text{-}NH_4^+.L^{-1}$ - is not limiting) and in the CBR continuous operation.

CONCLUSIONS

The respiration rate is a valuable tool for characterization of ammonia and nitrite oxidizing bacteria in a nitrifying biofilm. The respiration rate can be considered as an indicator for the nitrification activity of a biofilm and used for identifying disturbances that affect process performance. Moreover, the specific respiration rate could be an useful tool for controlling nitrification reactor performance, in particular those due to a pH effect.

Further work using genetic probes is underway in order to asses the relative amount of both nitrifying populations and compare the change in population dynamics with the evolution of specific respiration activities.

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